TABLE II

Marker Profile of Breast Carcinoma Progression

	Percent Positive Markers Studied:		
Tumor Type	p53	TSP-1	Microvasc.
Low-grade DCIS[1] (n=26)	0	89	4
High-grade DCIS (n=36)	31*	82	23
Invasive - LN(-)[2] (n=19)	47	32*	53
Invasive - LN(+)[3] (n=17)	82*	0	100*

* = $p < 0.05$ for paired one-tailed t-test, comparing the designated group with the group immediately above it in the Table

1 = ductal carcinoma *in situ*

2 = lymph node negative

3 = lymph node positive

These results demonstrated that nuclear localization of p53, decreased thrombospondin 1 expression, and increased microvascularization were significantly correlated with increased invasiveness of primary breast cancer, increased metastasis, and poorer prognosis for breast cancer patients whose tumors had these markers. To determine whether disease progression was linked not only to the incidence, but also the degree of marker expression as well, the intensity of staining of the markers as determined above by immunohistochemistry or image analysis was plotted *versus* tumor histology for the four histological subsets described above. These results are shown in Figures 1A through 1C. These results demonstrate a distinct pattern of differences in intensity and degree of expression of the three tumor markers assayed above. These results show that nuclear p53 accumulation and the number of tumor microvessels increased in both the transition from low-grade DCIS to high-grade DCIS, and also in the transition from invasive tumors without evidence

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of metastatic spread to invasive tumors having metastasis-positive lymph node involvement Figures 1A and 1B). TSP-1 expression showed a significant decline in intensity between high-grade DCIS and invasive cancer prior to metastatic spread.

These results are also shown graphically in Figure 2, where the increase in p53 nuclear accumulation and microvascularization and decrease in TSP-1 expression with tumor progression is shown. These data suggest that a coordinated relationship existed between nuclear p53 accumulation and angiogenesis, while TSP-1 expression was inversely correlated with these two factors. Invasion and metastasis in breast cancer were associated in a statistically-significant way with acquisition of dysfunctional p53 (as evidenced by nuclear accumulation), decreased TSP-1 expression, and increased angiogenesis.

EXAMPLE 2

Tumor Prognostic Index

The results obtained in the assays described in Example 1 above were used to construct a prognostic (risk) index relating tumor progression and increasingly poorer disease prognosis with positive marker results, graded by intensity of immunohistochemical staining of each of the tumor markers.

The IHC scores obtained in Example 1 were used to construct a tumor progression/prognosis (risk) index as follows. Scores for each of the markers were associated with a integer index scale from +1 to -4. This index scale was constructed for each marker based on the following IHC staining results as follows. The p53 nuclear accumulation score was derived from the percentage of cell staining with anti-p53 antibody multiplied by (1 + intensity of staining), using the intensity of staining and positive control cells described in Example 1. The TSP-1 score was derived from IA data obtained as described in Example 1, as weighted based on the percentage of cells staining positively for TSP-1. Angiogenesis was scored as greatest number of microvessels per field stained with anti-CD31 antibody, after a minimum scan of 10 fields. These scores and their associated weighted index scores are described below in Table III.

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TABLE III

p53, TSP-1 and Angiogenesis Indices

Index	p53 Score	Thrombospondin Score	Microvascularization Score
1	0-30	>30	0-30
0	31-60	25-29	31-70
-1	61-90	20-24	71-85
-2	91-120	15-19	86-100
-3	121-150	10-14	101-123
-4	>150	0-9	>123

The tumor prognosis (risk) index is then prepared by the sum of the index scores for p53 accumulation, TSP-1 expression and angiogenesis (microvascularization), with poor prognosis being determined for tumors having an summed index score of -5 or less.

The efficacy the prognostic (risk) index was assessed using Log rank tests on survival *versus* index score. The significance of the index scores on survival are shown in Table IV. In this table, it can be seen that a statistically-significant difference was observed in survival between patients having tumors with a summed index score $\geq$-5 when compared with patients having tumors with a summed index score $\leq$-6. Similarly, a statistically-significant difference was observed in survival between patients having tumors with a summed index score $\geq$-6 when compared with patients having tumors with a summed index score $<$-7. Finally, there was a statistically-significant difference was observed in survival between patients having tumors with a summed index score $\geq$-7 when compared with patients having tumors with a summed index score $<$-8.

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